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missing requirements under 35 U.S.C. 371 in the United States
Designated/Elected Office (DO/EO/US); Copy of Notification to comply with
requirements for patent applications containing nucleotide sequence and/or amino
acid sequence disclosures; Declaration; Statement under 37 C.F.R. §§ 1.821(f) &
(g); Paper copy of sequence listing; CRF of sequence listing; Amendment with
version with markings to show changes made (10 pages); Petition for extension of
time (in duplicate); and Check No. **33** in the amount of \$110.00.

Invention: SELECTING ANIMALS FOR PARENTALLY IMPRINTED
TRAITS

Applicant(s): ANDERSSON et al.

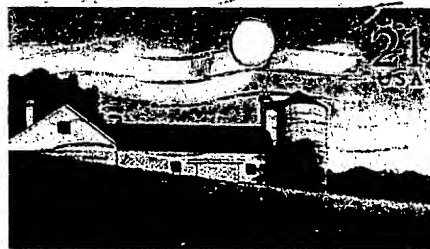
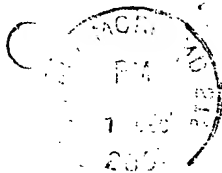
Filing Date: June 15, 2001

Serial No.: 09/868,732

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Client/Matter Docket No.: 2183/4951US

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PATENT
4951US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

ANDERSSON et al.

Serial No.: 09/868,732

Filed: June 15, 2001

For: SELECTING ANIMALS FOR
PARENTALLY IMPRINTED TRAITS

Examiner: To be assigned

Group Art Unit: To be assigned

Attorney Docket No.: 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label
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Date of Deposit with USPS: November 1, 2001

Person making Deposit: Daniel Thatcher

AMENDMENT

Box Non-Fee Amendment
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced patent application on the merits, entry of the amendments as set forth herein is respectfully solicited.

Pursuant to 37 C.F.R. §§ 1.121(b)(1)(i), please delete paragraphs [0019], [0021], [0023], [0053], [0054], and [0069] of the substitute specification mailed by the applicants on September 17, 2001, and replace the same with the like-numbered replacement paragraphs [0019], [0021], [0023], [0053], [0054], and [0069] set forth herein. In compliance with 37 C.F.R. §§ 1.121(b)(1)(ii), the above-referenced replacement paragraphs are submitted in clean form below. A version of the replacement paragraphs with markings to show changes made relative to the previous version of such

paragraphs is appended hereto under the title “VERSION WITH MARKINGS TO SHOW CHANGES MADE,” pursuant to 37 C.F.R. §§ 1.121(b)(1)(iii).

IN THE SPECIFICATION:

[0019] Figure 6: Nucleic acid sequences of contig 1 (SEQ ID NO:10), contig 2 (SEQ ID NO:11), contig 3 (SEQ ID NO:12), contig 4 (SEQ ID NO:13), contig 5 (SEQ ID NO:14), contig 6 (SEQ ID NO:15), contig 7 (SEQ ID NO:16), contig 8 (SEQ ID NO:17), contig 9 (SEQ ID NO:18), contig 10 (SEQ ID NO:19), contig 19 (SEQ ID NO:20), contig 20 (SEQ ID NO:21), contig 21 (SEQ ID NO:22), contig 22 (SEQ ID NO:23), contig 23 (SEQ ID NO:24), contig 24 (SEQ ID NO:25), contig 25 (SEQ ID NO:26), contig 26 (SEQ ID NO:27), contig 27 (SEQ ID NO:28), contig 28 (SEQ ID NO:29), contig 29 (SEQ ID NO:30), contig 30 (SEQ ID NO:31), contig 31 (SEQ ID NO:32), contig 32 (SEQ ID NO:33), contig 33 (SEQ ID NO:34), contig 34 (SEQ ID NO:35), contig 35 (SEQ ID NO:36), contig 36 (SEQ ID NO:37), contig 37 (SEQ ID NO:38), contig 38 (SEQ ID NO:39), contig 39 (SEQ ID NO:40), contig 40 (SEQ ID NO:41), contig 41 (SEQ ID NO:42), contig 42 (SEQ ID NO:43), contig 43 (SEQ ID NO:44), contig 44 (SEQ ID NO:45), contig 45 (SEQ ID NO:46), contig 46 (SEQ ID NO:47), contig 47 (SEQ ID NO:48), contig 48 (SEQ ID NO:49), contig 49 (SEQ ID NO:50), contig 50 (SEQ ID NO:51), contig 51 (SEQ ID NO:52), contig 52 (SEQ ID NO:53), contig 53 (SEQ ID NO:54), contig 54 (SEQ ID NO:55), contig 55 (SEQ ID NO:56), contig 56 (SEQ ID NO:57), contig 57 (SEQ ID NO:58), contig 58 (SEQ ID NO:59), contig 59 (SEQ ID NO:60), contig 60 (SEQ ID NO:61), contig 61 (SEQ ID NO:62), contig 62 (SEQ ID NO:63), contig 63 (SEQ ID NO:64), contig 64 (SEQ ID NO:65), contig 65 (SEQ ID NO:66), contig 66 (SEQ ID NO:67), contig 67 (SEQ ID NO:68), contig 68 (SEQ ID NO:69), contig 69 (SEQ ID NO:70), contig 70 (SEQ ID NO:71), contig 71 (SEQ ID NO:72), contig 72 (SEQ ID NO:73), contig 73 (SEQ ID NO:74), contig 74 (SEQ ID NO:75), contig 75 (SEQ ID NO:76), contig 76 (SEQ ID NO:77), contig 77 (SEQ ID NO:78), contig 78 (SEQ ID NO:79), contig 79 (SEQ ID NO:80), contig 80 (SEQ ID NO:81), contig 81 (SEQ ID NO:82), contig 82 (SEQ ID NO:83), contig 83 (SEQ ID NO:84), contig 84 (SEQ ID NO:85), contig 85 (SEQ ID NO:86), contig 86 (SEQ ID NO:87), contig 87 (SEQ ID NO:88), contig 88 (SEQ ID NO:89), contig 89 (SEQ ID NO:90), contig 90 (SEQ ID NO:91), contig 91 (SEQ

ID NO:92), contig 92 (SEQ ID NO:93), contig 93 (SEQ ID NO:94), contig 94 (SEQ ID NO:95), contig 95 (SEQ ID NO:96), contig 96 (SEQ ID NO:97), contig 97, (SEQ ID NO:98), contig 98 (SEQ ID NO:99), contig 99 (SEQ ID NO:100), contig 100 (SEQ ID NO:101), contig 101 (SEQ ID NO:102), contig 102 (SEQ ID NO:103), contig 103 (SEQ ID NO:104), contig 104 (SEQ ID NO:105), contig 105 (SEQ ID NO:106), contig 106 (SEQ ID NO:107), contig 107 (SEQ ID NO:108), contig 108 (SEQ ID NO:109), contig 109 (SEQ ID NO:110), contig 110 (SEQ ID NO:111), contig 111 (SEQ ID NO:112), contig 112 (SEQ ID NO:113), contig 113 (SEQ ID NO:114), contig 114 (SEQ ID NO:115), and contig 115 (SEQ ID NO:116) derived from BAC-PIGF2-1, which was shotgun sequenced using standard procedures and automatic sequencers.

[0021] Figure 8: Nucleic acid sequences of contig 1 (SEQ ID NO:117), contig 2 (SEQ ID NO:118), contig 3 (SEQ ID NO:119), contig 4 (SEQ ID NO:120), contig 5 (SEQ ID NO:121), contig 6 (SEQ ID NO:122), and contig 7 (SEQ ID NO:123) derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1), which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

[0023] Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals. Polymorphisms 1 through 4 occur in contig 3 (SEQ ID NO:12), polymorphisms 5 through 23 occur in contig 4 (SEQ ID NO: 13), polymorphisms 24 through 28 occur in contig 10 (SEQ ID NO:19), polymorphism 29 occurs in contig 57 (SEQ ID NO:58), polymorphism 20 occurs in contig 95 (SEQ ID NO:96), and polymorphism 31 occurs in contig 105 (SEQ ID NO:106).

[0053] Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' (SEQ ID NO:1) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ ID NO:2)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described²⁴. The BAC DNA was linearized with *EcoRV* and purified

with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described²⁵. The final concentration of the probe in the hybridisation mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

[0054] About two μg of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ ID NO:3) and R: 5' -Fluorescein- CTACAAGCTGGGCTCAGGG-3' (SEQ ID NO:4)) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analyses were done with the Cri-Map software²⁶.

[0069] *Marker genotyping:* Primer pairs utilised for PCR amplification of microsatellite markers are as described¹⁹. Marker genotyping was performed as previously described²⁰. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described^{1,12}. The LAR test for the IGF2 SNP was developed according to Baron et al.²¹ using a primer pair for PCR

amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3'(SEQ ID NO:5); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3')(SEQ ID NO:6) and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCAG-3'(SEQ ID NO:7); 5' -HEX-CGCCCCAGCTGCCCCCAA-3'(SEQ ID NO:8); 5' -CCTGAGCTGCAGCAGGCCAG-3' (SEQ ID NO:9)).

REMARKS

No new matter has been added. The Applicants again request entry of the amendments as set forth herein prior to examination of the application on the merits.

Respectfully submitted,

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Telephone: (801) 532-1922

Date: May 12, 2005

VERSION WITH MARKINGS TO SHOW CHANGES MADE

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[0019] Figure 6: Nucleic acid sequences of contig 1 (SEQ ID NO:10), contig 2 (SEQ ID NO:11), contig 3 (SEQ ID NO:12), contig 4 (SEQ ID NO:13), contig 5 (SEQ ID NO:14), contig 6 (SEQ ID NO:15), contig 7 (SEQ ID NO:16), contig 8 (SEQ ID NO:17), contig 9 (SEQ ID NO:18), contig 10 (SEQ ID NO:19), contig 19 (SEQ ID NO:20), contig 20 (SEQ ID NO:21), contig 21 (SEQ ID NO:22), contig 22 (SEQ ID NO:23), contig 23 (SEQ ID NO:24), contig 24 (SEQ ID NO:25), contig 25 (SEQ ID NO:26), contig 26 (SEQ ID NO:27), contig 27 (SEQ ID NO:28), contig 28 (SEQ ID NO:29), contig 29 (SEQ ID NO:30), contig 30 (SEQ ID NO:31), contig 31 (SEQ ID NO:32), contig 32 (SEQ ID NO:33), contig 33 (SEQ ID NO:34), contig 34 (SEQ ID NO:35), contig 35 (SEQ ID NO:36), contig 36 (SEQ ID NO:37), contig 37 (SEQ ID NO:38), contig 38 (SEQ ID NO:39), contig 39 (SEQ ID NO:40), contig 40 (SEQ ID NO:41), contig 41 (SEQ ID NO:42), contig 42 (SEQ ID NO:43), contig 43 (SEQ ID NO:44), contig 44 (SEQ ID NO:45), contig 45 (SEQ ID NO:46), contig 46 (SEQ ID NO:47), contig 47 (SEQ ID NO:48), contig 48 (SEQ ID NO:49), contig 49 (SEQ ID NO:50), contig 50 (SEQ ID NO:51), contig 51 (SEQ ID NO:52), contig 52 (SEQ ID NO:53), contig 53 (SEQ ID NO:54), contig 54 (SEQ ID NO:55), contig 55 (SEQ ID NO:56), contig 56 (SEQ ID NO:57), contig 57 (SEQ ID NO:58), contig 58 (SEQ ID NO:59), contig 59 (SEQ ID NO:60), contig 60 (SEQ ID NO:61), contig 61 (SEQ ID NO:62), contig 62 (SEQ ID NO:63), contig 63 (SEQ ID NO:64), contig 64 (SEQ ID NO:65), contig 65 (SEQ ID NO:66), contig 66 (SEQ ID NO:67), contig 67 (SEQ ID NO:68), contig 68 (SEQ ID NO:69), contig 69 (SEQ ID NO:70), contig 70 (SEQ ID NO:71), contig 71 (SEQ ID NO:72), contig 72 (SEQ ID NO:73), contig 73 (SEQ ID NO:74), contig 74 (SEQ ID NO:75), contig 75 (SEQ ID NO:76), contig 76 (SEQ ID NO:77), contig 77 (SEQ ID NO:78), contig 78 (SEQ ID NO:79), contig 79 (SEQ ID NO:80), contig 80 (SEQ ID NO:81), contig 81 (SEQ ID NO:82), contig 82 (SEQ ID NO:83), contig 83 (SEQ ID NO:84), contig 84 (SEQ ID NO:85), contig 85 (SEQ ID NO:86), contig 86 (SEQ ID NO:87), contig 87 (SEQ ID NO:88), contig 88 (SEQ ID NO:89), contig 89 (SEQ ID NO:90), contig 90 (SEQ ID NO:91),

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